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Optimization of enzymatic process for preparation of absorbent cotton[a]

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An ecofriendly process has been developed for preparation of absorbent cotton using short staple fibres of cotton. The crude enzyme extract of solid state fermentation has been employed for absorbent cotton preparation and the treatment conditions are optimized. Five gram of short staple fibre is used in each treatment. Under optimized solid state fermentation conditions, such as fungal strain (*P. flabellatus*), substrate composition (banana pseudo stem, cottonseed hulls and cottonseed meal in the ratio of 60:30:10) and fermentation period (5 days), the absorbency recorded is found 7 s. In another experiment, process parameters of single bath enzymatic scouring and bleaching process are also optimized. Under optimized process conditions, such as enzyme extract (30 %), temperature (60ºC), time (40 min), *p*H (9.0) and wetting agent (0.1%), the absorbency is found 2 s and whiteness index is 31.5 (CIE method). The pectinase and laccase activity recorded in the enzyme extract is found to be 28.1 and 6 units per milliliter respectively. The enzymes remain active at different temperature and *p*H tested. The characterization using scanning electron microscope (SEM) reveals the fibre surface modification in the enzyme treated cotton.

Keywords: Absorbency, Absorbent cotton, Banana pseudo stem, Cottonseed hull, *P. flabellatus*, Short staple cotton, Solid state fermentation, Whiteness index

1 Introduction

Absorbent cotton is the fibrous material which finds application in absorbing body fluids during surgery, wound healing treatment, earbuds, cosmetic wipes preparation, etc. The raw material required for manufacturing of absorbent cotton is either short staple fibre or non-spinnable cotton. The short staple cotton (*G. arboretum*) is found ideal for absorbent cotton preparation. According to an estimate, India needs about 2 million bales (170 kg) of absorbent cotton every year[1]. Conventionally, the absorbent cotton is prepared by scouring cotton at higher temperature (100-115 °C) and pressure under alkaline conditions followed by bleaching with hydrogen peroxide at higher *p*H to improve the whiteness of cotton. In recent days, industries combine scouring and bleaching, since both the steps require higher temperature and *p*H (10.5). The generation of toxic effluents due to this process is a great concern these days due to strict environment compliance required for industries.

To overcome this issue, researchers are paying attention on development of ecofriendly and economically competitive process using enzyme technology as an alternative to conventional high energy, water and chemical consuming process in the preparation of absorbent cotton[2]. Commercial pectinase was used in the preparation of absorbent cotton[3]. Single bath scouring and bleaching of cotton was carried out using commercial pectinase and hydrogen peroxide respectively[4]. Solid state fermentation was employed for the production of industrial enzymes such as pectinase, laccase, etc. using different agro-residues[5-7]. A low-cost agro-waste (banana peel) was used for production of high activity pectinase by a fungus (*Aspergillus niger*)[8]. However, literature related to the application of crude enzyme prepared from agro-residues in absorbent cotton preparation is still lacking. Considering this, the present study was attempted to develop a crude enzyme extract from solid state fermentation of agro-residues so as to replace the toxic chemicals used in scouring and bleaching process for preparing the absorbent cotton.

2 Materials and Methods

2.1 Short Staple Fibres

Short staple cotton fibres (Phule Dhanwantry var.) were procured from ICAR- Central Institute for

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Cotton Research, Nagpur. The fibre was opened through trash separator. The fibre quality of procured cotton was found to be strength 16.3 g/tex, length 20.6 mm, micronaire 6.9 and whiteness index 16.9.

2.2. Microorganism

The test microorganisms, such as *Pleurotus flabellatus*, *Bacillus stearothermophilus* and *Phanerocheate chrysosporium,* were grown in malt extract broth (1X) at 30ºC for 48 h and maintained in malt extract agar slant at 4ºC.

2.3 Substrates for Solid State Fermentation

The agro residues such as banana pseudo stem wastes, orange peel, cottonseed hulls and cottonseed meal, were purchased from market. These substrates were dried in oven, powdered using mixer and passed through 1mm sieve. These fine powdered substrates were used for solid state fermentation.

2.4 Selection of Microbial Strain

The microbial strains, viz. *B. stearothermophilus*, *P. flabellatus*, *P. chrysosporium* and the combinations of three were screened for solid state fermentation. Solid state fermentation was carried out in one liter conical flask containing 100 gram of banana pseudo stem. Eighty milliliter of distilled water was added to maintain initial moisture content of 80 % (v/w). The moistened substrate was autoclaved at 121º C for 15 lbs/in^2 for 20 min. After cooling, the substrates were inoculated with 5 % of 48 h old culture of test strains and incubated for one week. The enzyme extract obtained from fermented substrates was used for absorbent cotton treatment as described below.

2.4.1 Enzyme Extraction

The enzyme was extracted by adding total one liter of distilled water in the fermented substrate, stirred for 30 min and filtered through muslin cloth. The enzyme extract was stored at 4° C until use.

2.4.2 Absorbent Cotton Treatment

The enzyme extract was used for absorbent cotton treatment. Five gram short staple cotton fibre was taken in 500 mL beaker to which 125 mL of enzyme extract and 125 mL of distilled water were added, thus 50 % (v/v) of enzyme extract were added in bath solution. Care was taken to observe that fibre is immersed completely in the bath. The beaker was kept in reciprocating water bath (Rivotek TC 344, Rivera, India) at 60ºC and 50 rpm for 30 min. After treatment, the sample was cooled and washed 4-5 times in running tap water, squeezed and removed water, dried in oven at 100º C for 4 h. The dried cotton was tested for absorbency (s) according to IS 2369-1967 after certain modification[9].

2.5 Optimization of Solid State Fermentation Process

The selected microbial strain was used for optimization of solid state fermentation. The substrates, such as banana pseudo stem, orange peel, cottonseed hulls and cottonseed meal, at different combinations (Table 1) were tested. The solid state fermentation, enzyme extraction and absorbent cotton treatment were carried out as described earlier. Under optimized substrate composition, the effect of various incubation periods (3, 5 and 7 days) of solid state fermentation process on absorbent cotton preparation was studied. The prepared cotton was tested for absorbency as described earlier. Each sample was replicated and the average value of each set of experiments was accounted.

2.6 Optimization of Absorbent Treatment Process Parameters

The process parameters, such as enzyme extract level, temperature, time and *p*H, were optimized for absorbent cotton preparation under standardized solid state fermentation conditions, such as microbial strain (*P. flabellatus*), substrate (banana pseudo stem: cottonseed hulls: cottonseed meal, 60: 30: 10), and incubation period (5 days). The material-to-liquor ratio was kept at 1: 50. Five gram of short staple cotton was taken in 250 mL of liquor solution in each treatment. The different levels of enzyme extract, such as 5, 10, 20, 30, 40 and 50 % were tested, keeping temperature 60 ºC, time 30 min and *p*H 7.0. The effect of different levels of temperature (40, 50, 60, 70 and 80 º C) was tested, keeping optimum level of 30 % enzyme extract, 30 min treatment time and *p*H 7. The various treatment time (10, 20, 30 and 40 min) was tested, keeping optimum level of 30 % enzyme extract, 60° C temperature and *p*H 7. The various levels of *p*H (5, 7 and 9) were tested, keeping

Table 1 ― Optimization of solid state fermentation substrate composition

Treatment code	Substrate composition			
	Banana pseudo stem	Orange peel	Cottonseed hulls	Cottonseed flour
1	0	60	30	10
2	10	50	30	10
3	20	40	30	10
4	30	30	30	10
5	40	20	30	10
6	50	10	30	10
7	60	0	30	10

optimum level of 30 % enzyme extract, 60 °C temperature and 40 min time. The samples were tested for absorbency and whiteness index (CIE, 2000)[4]. Each treatment was replicated and the average value of each set of experiments was taken into account.

2.7 Effect of Non-ionic Wetting Agent

The effect of non-ionic wetting agent (Empi 100®) at different levels (0.05, 0.1 and 0.2 % v/v) was tested on absorbent cotton. The other conditions were kept as similar as described earlier. The samples were tested for absorbency and whiteness index. The other parameters tested were *p*H, water holding capacity (g/g) and wax content (%)[1, 4].

2.8 Characterization of Crude Enzyme

The pectinase and laccase activity were estimated in crude enzyme extract. The pectinase activity was determined by estimation of polygalactouronase activity and analysis of reducing sugars released from citrus pectin during the reaction[10]. One unit of enzymatic activity (U) was defined as the amount of enzyme which releases one µmol of galactouronic acid per minute. Laccase activity was estimated using syringaldazine as substrate [Sigma-Aldrich (EC 1.10.3.2)]. In this method, 0.3 mL of syringaldazine solution was mixed with 0.5 mL of enzyme extract and 2.2 mL of deionized water, and the increase in absorbance at 530 nm for 10 min was recorded using UV-Vis Spectrophotometer. One unit of enzyme activity (U) was defined as the change in absorbency of 0.001 at 530 nm, per min at *p*H 6.5 and temperature 30 °C.

The effect of *p*H (5, 7 and 9), temperature (40, 60 and 80 °C), non-ionic wetting agent (0.2 %) and hydrogen peroxide (Emparta®, Merck India) (2.5%) on pectinase and laccase activity was determined. To determine the effect of *p*H, the enzyme extract was adjusted for the required *p*H with 0.1 N HCl/ NaOH and incubated at 30 °C for 1 h. To study the effect of temperature on enzyme activity, the enzyme extract was kept in water bath at the above mentioned different temperature for 1h. The non-ionic wetting agent and hydrogen peroxide were added separately in crude enzyme and incubated at 30 °C for 1 h. A control was maintained with no addition at natural *p*H (6.5), 30 °C temperature and 1h treatment time.

2.9 Characterization of Absorbent Cotton Prepared using Enzymatic Process

The absorbent cotton produced using the developed method was characterized using FTIR spectra and SEM analysis. The samples (raw cotton and absorbent cotton) were processed with KBr to make a pellet. Spectra of the pellet were recorded in the transmission mode between wave numbers 500 cm^{-1} and 4500 cm^{-1} using a resolution of 20 kHz scan speed in Shimadzu IR Prestige 21 analyzer. The surface changes in the samples (raw cotton and absorbent cotton) was observed using SEM (Philips XL-30) with an accelerating voltage of 12 kV. The sample was coated with a thin layer of conducting material (gold/palladium) using a sputter coater.

3 Results and Discussion

As per Indian Pharmacopoeia, absorbent cotton should have absorbency of < 10 s, water holding capacity > 23 g/g of fibre, sulphated ash < 0.5 % and *p*H 5-8 (ref. 1). The absorbency is inversely related to sinking time (s), which means lower sinking time implies higher absorbency. Enzymatic scouring and bleaching of cotton is a viable alternative method to conventional alkali method which helps in significant reduction in release of toxic chemicals in to the environment[3]. Solid state fermentation is extensively used for isolation of enzymes and other bio-products for industrial applications[11, 12]. The enzymes involved in scouring and bleaching process are pectinase and laccase respectively. Among the different substrates, agro-residues are the cheapest and largely available source for production of industrially used enzymes especially pectinase and laccase enzymes[12].

In this study, crude enzyme for absorbent cotton treatment is extracted followed by solid state fermentation of agroresidues. Initially, the microbial strains, viz. *B. stearothermophilus*, *P. flabellatus*, *Phaenerocheate chrysoporium* and the combinations of the three cultures are screened for solid state fermentation and subsequent enzymatic treatment of cotton. Banana pseudostem has been used as substrate for solid state fermentation. The results showed that the absorbency (10 s) was higher in *P. flabellatus* enzyme extract treated cotton as compared to other treatments [Fig. 1 (a)]. *P. chrysosporium* enzyme extract does not show any absorbency. Hence, in the further study, *P. flabellatus* was used for solid state fermentation.

To find out the effect of solid state fermentation substrate on absorbency of cotton, different agroresidues, viz. banana pseudostem, orange peel, cottonseed hulls and cottonseed meal are evaluated (Table 1). It is observed that the absorbency is higher

(7 s) in treatment 7 (banana pseduostem, orange peel, cottonseed hulls and cottonseed meal, 60:0:30:10) followed by treatment 5 (banana pseduostem, orange peel, cottonseed hulls and cottonseed meal in the ratio 40:20:30:10) where absorbency is 10 s [Fig. 1(b)]. In the treatments 1, 2 and 3, where orange peel is added as major component, the absorbency of cotton is not detected. The higher absorbency recorded in the substrate ratio 60:0:30:10 might be due to high pectin content in banana pseudostem. The solid state substrate composition (banana pseudostem, cottonseed hulls and cottonseed meal 60:30:10) is chosen for further experiments. The effect of solid state fermentation period (incubation time) on absorbency of cotton is tested. The results show that the absorbency is highest (7 s) in incubation period of 5 days as compared to other treatments [Fig. 1(c)]. The incubation of three days does not show any absorbency in treated cotton. Hence, the solid state fermentation conditions such as microbial strain (*P. flabellatus*), substrate composition (Banana pseudostem, cottonseed hulls and cottonseed meal, 60:30:10) and incubation period (5 days) are selected for subsequent absorbent cotton process parameters optimization study.

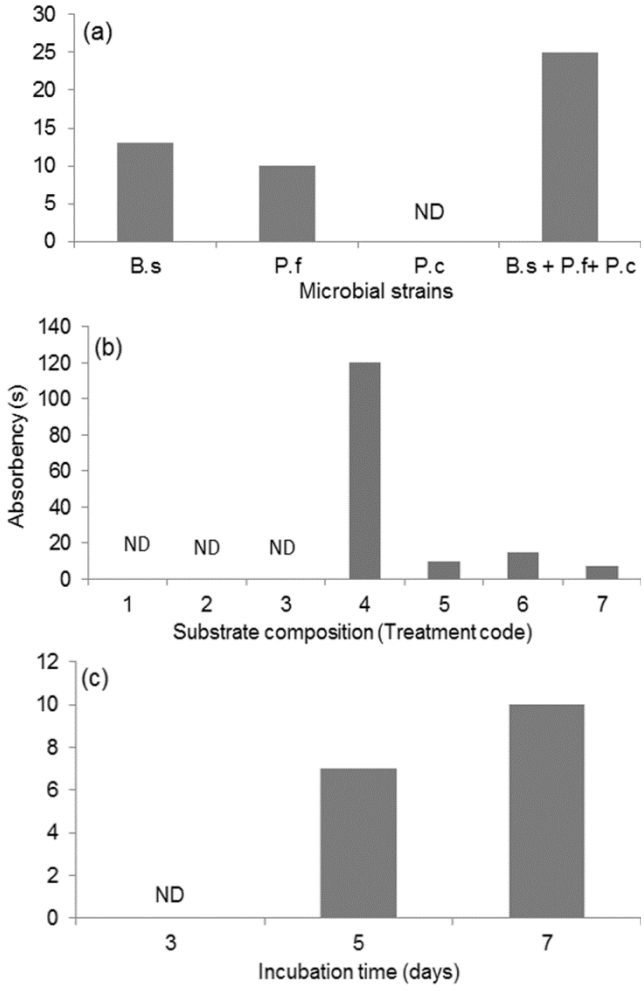

Fig. 1 — Optimization of solid state fermentation process (a) microbial strains, (b) substrate composition and (c) incubation time on absorbency of short staple cotton [ND – not detected, B.s – *Bacillus stearothermophilus*, P.f- *Pleurotus flabellatus* and P.c- *Phanerocheate chrysosporium*]

Single bath scouring and bleaching is followed for absorbent cotton preparation. The process parameters such as enzyme extract level, temperature, time, *p*H and wetting agent level are optimized for absorbent cotton preparation using the indices absorbency and whiteness index. Among different enzyme extract level tested, the enzyme extract level of 30 % has higher absorbency (10 s) among all other treatments [Fig. 2(a)]. The enzyme extract levels of 5% and 10 % do not produce any absorbency in the treated cotton. The whiteness index shows decreasing trend with the increase in concentration of enzyme extract. The impurities in the crude enzyme might interfere with whiteness index at higher concentration. Hence, 30% of enzyme extract is used for further experiments. The optimum temperature for enzymatic process of absorbent preparation is found to be 60 °C. The absorbency and whiteness index observed at this temperature are 7 s and 15 respectively [Fig. 2(b)]. The process temperatures 70° and 80 °C do not show any absorbency in the treated cotton. Thus, the optimum temperature of 60° C is kept for subsequent experiments. The effect of different time on absorbent cotton preparation is evaluated. The results show that the process time of 40 min is the optimum treatment time [Fig. 2(c)]. The absorbency and whiteness index recorded at this treatment time are 6 s and 20.4 respectively. The further increase in time has no positive effect on absorbency and whiteness index. For subsequent experiments, the treatment time of 40 min is considered optimum for absorbent cotton preparation. The optimum *p*H for absorbency and whiteness index in absorbent cotton is found as 9.0. This indicates the alkaline nature of pectinase and laccase enzymes present in crude extract. The absorbency and whiteness index observed at *p*H 9.0 are 2 s and 27.4 respectively [Fig. 2(d)]. Hence, *p*H, 9.0 (optimum) is kept for further experiments.

In order to find out the effect of non-ionic wetting agent on absorbent cotton preparation, different levels of wetting agent are tested. The absorbency and

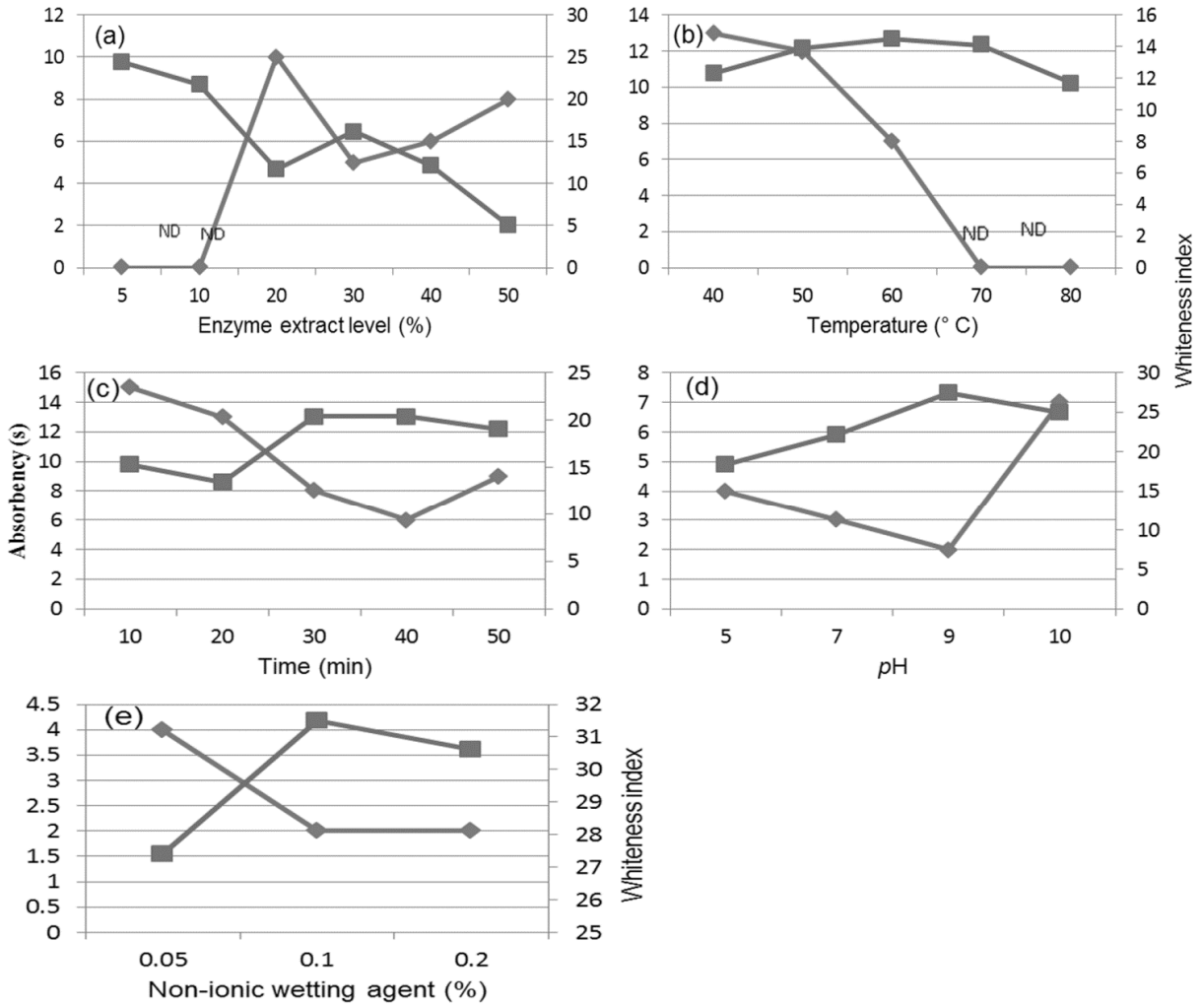

Fig. 2 — Optimization of absorbent cotton preparation process parameters (a) enzyme extract level, (b) temperature, (c) time, (d) *p*H and (e) wetting agent on absorbent cotton preparation [absorbency (♦), whiteness index (■), and ND (not detected)

whiteness index has increased by 2 s and 31.5 respectively at 0.1 % (v/v) non-ionic wetting agent [Fig. 2(e)]. In a similar study, 0.5 % of wetting agent is added on the weight of fibre to get the desired results[3]. Thus, the optimized process conditions obtained in the study are enzyme extract (30%), temperature (60° C), time (40 min), *p*H (9.0) and non-ionic wetting agent (0.1 %). Table 2 shows the properties of absorbent cotton prepared by optimized enzymatic process in comparison with Indian Pharmacopeia standards. It shows that the properties of absorbent cotton, viz. absorbency, water holding capacity and ash content, meet the standards of Indian Pharmacopeia. The absorbency, water holding capacity (g/g), ash content (%), whiteness index and wax content (%) of the absorbent cotton prepared by the optimized process are 2, 26.3, 0.25, 31.5 and 0.84 respectively.

Table 2 — Properties of enzymatically prepared absorbent cotton

Parameter	Enzymatically prepared absorbent cotton	Indian pharmacopeia standard
Absorbency, s	2.6	<10
Water holding capacity, g/g	26.3	23
Sulphated ash content, %	0.25	<0.5
*p*H	6.5	5-8
Whiteness index	31.5	-
Wax content, %	0.84	-

The raw cotton contains 86 – 96 % cellulose and 6 - 14 % of non-cellulosic impurities. The pectin and the waxes are the major impurities present in cotton. Pectin binds with waxes on the outer layer of fibre and is responsible for non-absorbent characteristics of cotton. Pectinase acts on pectin layer in fibre and releases waxes from the surface of cotton. This action

improves the absorbent characteristics of cotton. The use of commercial pectinase enzymes for absorbent cotton preparation is well reported[13-15]. In this study, the crude enzyme used for absorbent cotton treatment has the pectinase activity of 28.1 U/mL (Table 3). Conventionally, hydrogen peroxide has been used for bleaching process. In this study, attempt has been made to evaluate the effect of enzyme extract on scouring as well as bleaching. Hence, the chemicals used for scouring and bleaching are totally avoided to develop a completely green process for absorbent cotton preparation. Laccase has been reported for bio-bleaching of cellulose and cellulosic products[10]. In the present study, the laccase activity in the crude enzyme extract is 6 U/ml (Table 3). A substantial increase in whiteness index from 16.9 to 31.5 is recorded in treated cotton.

Different *p*H (5, 7 and 9), temperature (40, 60 and 80), wetting agent (0.2 %) and H_2O_2 (2.5 %) are evaluated for their effects on activities of pectinase and laccase in crude enzyme extract (Table 3). The pectinase and laccase activities of crude enzyme extract (without any treatment) are found 28.1 and 6 units/ml respectively. The increased pectinase activity is observed in different *p*H, wetting agent (0.2 %) and H_2O_2 (2.5 %). The pectinase activity is less at temperature higher than 30 °C, while the activity at temperature 60 °C is found more than that at 40°C and 80°C. The laccase activity is less in all the treatments except at *p*H 5, where the laccase activity is recorded as 8.0 U/mL. No laccase activity is observed in 0.2 % wetting agent. The results show that the crude enzyme possesses relatively higher pectinase activity than laccase. The pectinase and laccase activities in crude enzyme extract are resilient to wide range of temperature and *p*H. Thus, the study throws light on possible involvement of pectinase and laccase activities in crude enzyme extract for improvement in absorbency and whiteness index in the treated cotton.

Table 3 — Characterization of crude enzyme

Treatment	Pectinase, U/mL	Laccase, U/mL
Crude enzyme (natural *p*H 6 .5)	28.1	6
*p*H 5	53.0	8
pH 7	40.4	4
*p*H 9	40.4	4
Temperature 40 °C	16.7	6
Temperature 60 °C	22.3	4
Temperature 80 °C	9.19	4
Wetting agent (0.2 %)	59.9	No activity
H_2O_2 (2.5 %)	43.4	4

The FTIR spectral analysis of treated cotton and raw cotton (Fig. 3) does not show any major differences in the spectrum pattern. Chung *et al.* [16] reported that FTIR spectrum in transmission mode of raw and scoured cotton fabrics are similar due to the fact that bulk compositions of the fabrics are similar. Waxes and pectins of non-cellulosic components are located on the rim of cotton fibres, also the quantity of these impurities is too small to be seen in the spectrum. In order to evaluate the surface modification of cotton fibre due to enzymatic treatment, SEM analysis has been carried out (Fig. 4). It is observed that the treated cotton [Fig. 4 (b)] is more swollen than raw cotton [Fig. 4(a)]. The twisted

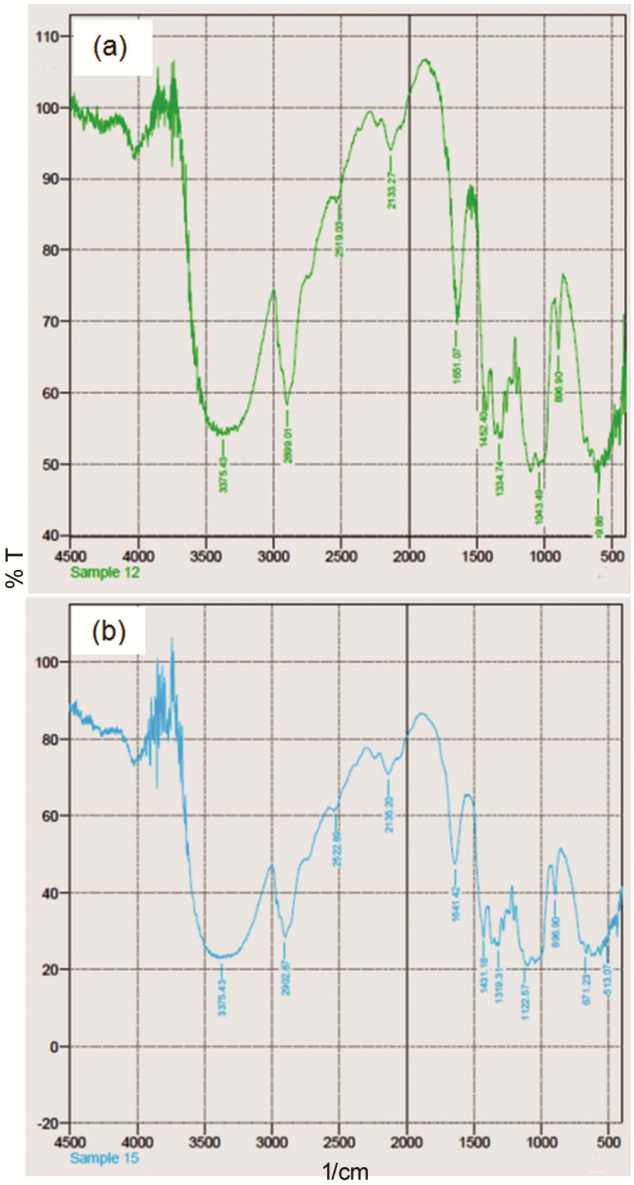

Fig. 3 — FTIR spectra of raw cotton (a), and treated cotton (b)

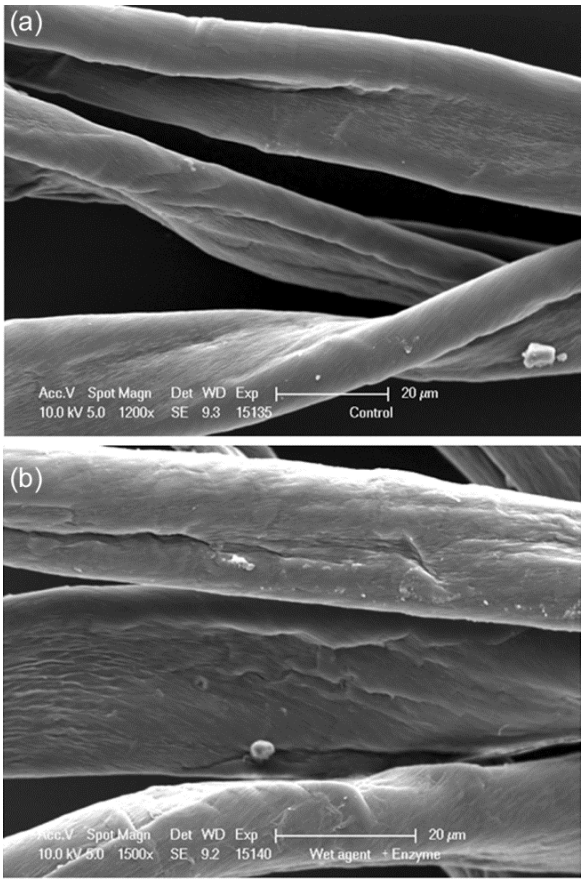

Fig. 4 — SEM micrograph of raw cotton (a), and treated cotton (b)

structure has been slightly converted into cylindrical in treated cotton. These properties can provide luster as well as water holding capacity. The outer layer of treated cotton is loosened and some abrasions areobserved. This might be due to the action of pectinase in the crude enzyme which results in improvement in absorbency of cotton.

4 Conclusion

In the present study, a solid state fermentation process is optimized to produce enzyme extract for absorbent cotton treatment. The optimized solid state fermentation process parameters are, fungal strain (*P. flabellatus*), substrate composition (banana pseudo stem, cottonseed hulls and cottonseed meal in the ratio of 60:30:10) and fermentation period (5 days). The pectinase and laccase activities recorded in the enzyme extract are found to be 28.1 and 6 units per milliliter respectively. Similarly, the process for preparing absorbent cotton using enzyme extract is also optimized. The optimized absorbent cotton treatment process conditions are enzyme extract (30 %), temperature (60 º C), time (40 min), *p*H (9.0) and wetting agent (0.1%). The absorbency and whiteness index recorded under optimized conditions are 2 s and 31.5 respectively.

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